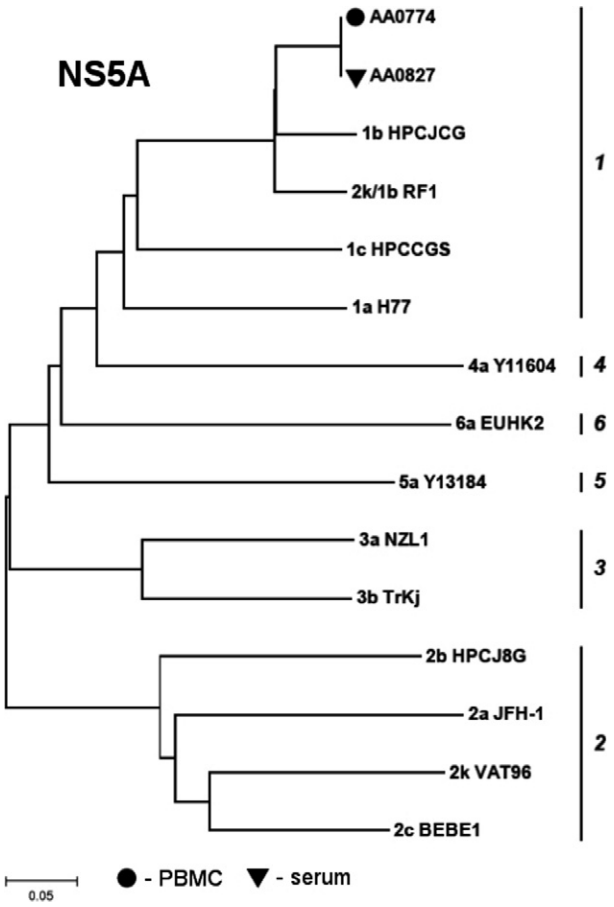

PP-146 **Establishment of highly efficient full-length HCV 1b genome cell culture system by inserting long distant structural fragment into replicon**

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Objectives: To establish a full-length genome of hepatitis C virus (HCV) 1b, the dominant strain in China, cell culture system for further study.
Methods: The 5′-end of half HCV-1b genome (5.2kb) was amplified from Chinese chronic hepatitis C patients' seral samples with the refined long distant RT-PCR technique. The full length recombinant plasmid of HCV 1b was constructed by inserting the long distant structural regions directly into HCV 1b replicon containing the non-structural regions. In vitro transcribed genomic HCV RNA was transfected into Huh7.5.1 cells by liposome-mediated method. The real time quantitative RT-PCR, Western Blot, inoculation of naïve Huh7.5.1 cells, immune fluorescence and titration of infectious HCV were used for identification of HCV replication and presence of infectious virion.
Results: The real time quantitative RT-PCR assay revealed the highest titer of HCV was 6.5×10^7 copies/mL in the cultural supernatants. While both Western Blot analysis and immune fluorescence confirmed the expression of HCV core protein in the transfected cells. Subsequent infection of naïve Huh7.5.1 cells with supernatant of HCV cell culture resulted in high levels of HCV proteins and RNAs.
Conclusions: These results demonstrate the successful establishment of a HCV 1b culture system by the new strategy of inserting long distant structural amplicon into replicon that produces infectious virus, which will allow the study of each aspect of the entire HCV life cycle and related studies.
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PP-147 **Construction of a chimeric GB virus B with hepatitis C virus NS2-NS4A**

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Background: HCV infection became a worldwide threat to human health, which can led to chronic hepatitis C, cirrhosis and hepatoma. As only human and chimpanzees are susceptible to hepatitis C virus, the progress of HCV research has been obstructed due to the absence of a reliable small primate animal model. GB virus B (GBV-B), being very close to hepatitis C virus (HCV) phylogenetically, can replicate effectively in vivio of common marmosets, that has been made an attractive surrogate virus for HCV replication study. It was reported that HCV NS2-NS3 protease, NS3 protease and NS4A complex are critical for virus maturation and replication. The construction of chimeric GB Virus B with Hepatitis C NS2-NS4A region can be used to develop a marmoset model for antiviral and immune studies.
Methods: RT-PCR and overlapping PCR were applied to complete jointing gene fragments and T7 transcription kit was used to produce chimeric GBV-B/HCV infectious RNA in vitro.
Results: A chimeric clone originating from GB virus B (GBV-B), in which GBV-B NS2-NS4A region are replaced by analogous sequence of the HCV genome, was constructed.
Conclusions: This chimeric clone lays the roots for a surrogate model of Hepatitis C virus, insights into HCV replication mechanism and further HCV NS3 epitope-based research.

PP-148 **Comparisons of molecular responses between recovery and chronic HCV infection from blood donors in Beijing and Guangdong, China**

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Background: Hepatitis C virus (HCV) infection is a major public health problem in China. However systematic studies conducted on mechanism of recovery and chronic infection with HCV are little known. A detailed study was carried out to investigate IFN-α and IFN-γ correlating to antibody reactivity and viral factors in the population of recovered and chronic HCV infection from blood donors.
Methods: 160 plasmas samples reactive with anti-HCV assays were collected from blood donors in Guangdong and Beijing. HCV antibody reactivity was presented as S/CO by at least three EIA assays. All samples were tested for ALT and viral load, confirmed by nested-PCR, and classified as three statuses of recovery (RNA–/Ab+), chronic (RNA+/Ab+) and false positive (RNA–/Ab–) infections. Productions of IFN-α and IFN-γ in the serum were also quantified by ELISA. The genotypes of HCV from chronic samples were phylogenetically analyzed with 5′-NCR sequences (215–218bp).
Results: 45 recovery, 76 chronic and 39 false positive of 160 HCV antibody positive plasmas were finally confirmed. The rate of recovered HCV infected individuals in blood donors was 37.2% proximately. 63 HCV strains were genotyped,